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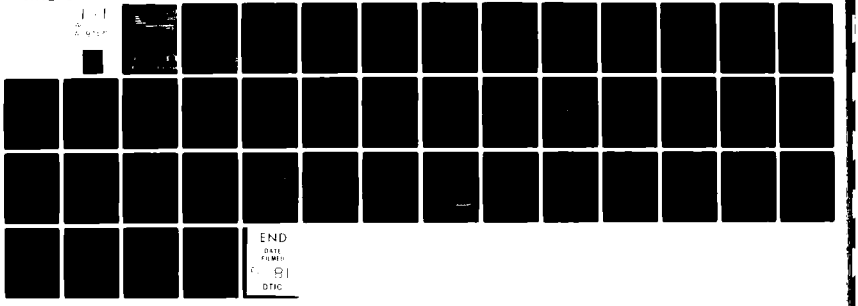

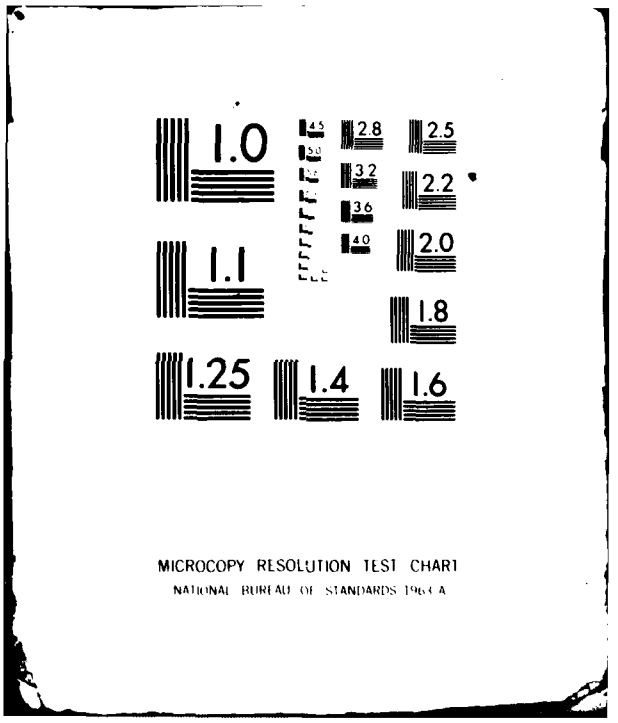
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CHARACTERIZATION OF THE CHEMICAL CONSTITUTION AND PROFILE OF PHARMACOLOGICAL ACTIVITY OF [illegible]

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(9) TECHNICAL REPORT NO. 2

(6) Characterization of the Chemical Constitution and Profile of Pharmacological Activity of PGE$_1$.

by

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PGEsub X

Studies on the Pharmacology of PGB_x

Unless otherwise indicated, the PGB_x used in these studies was supplied by Dr. H.W. Shmukler, Biochemistry Branch, ACSPD, Naval Air Development Center,* as a dark amber oily liquid or an alcoholic solution containing 155.6 mg of PGB_x acid per ml. It was converted to the water-soluble sodium salt by us and the freeze dried salt was stored at 5°C in a dessicator. For all biological studies, PGB_x-Na was dissolved in appropriate volumes of Sorenson's phosphate buffer (0.0667 M), pH 7.4 or saline, and passed through a cellulose triacetate membrane filter (0.2 micron pore size) into sterile stoppered serum vials. All pharmacological studies were performed using PGB_x-Na although the doses and concentrations are expressed in terms of PGB_x acid.

As described in the original and renewal proposals and consistent with the goals of the project we have a) established upper dosing limits for animal experiments based on an assessment of the acute toxicological profile of PGB_x,+ b) developed an organ-level assay of PGB_x activity based upon PGB_x's ability to enhance isoproterenol-induced contractility in isolated right atrial muscle of the guinea pig heart, c) partially characterized a long lasting inhibitory effect of acute and chronic PGB_x administration on the vasoconstricting action of sympathetic nerve stimulation — an effect that resembles that of PGE_1, d) evaluated the effects of PGB_x on blood pressure and heart rate of conscious unrestrained animals, and e) developed a cardiac function model in dogs that allows for a determination of PGB_x's influence on functional

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*This sample was described by H.W.S. as Fraction 2 (Sephadex LH20 column separation) of preparation No. 28.

+These observations were described in the Progress Report and Renewal Application dated 2 October 1979 and Technical Report No. 1 dated 30 April 1980 and will not be reviewed again here.

deficits induced by coronary insufficiency. This technique focuses on PGB_x's actions on selected cardiac tissues, viz., SA node, Purkinje system and ventricular myocardium.

Influence of PGB_x on cardiac contractility changes induced by inotropic drugs. We have examined the effects of PGB_x on the responsiveness of spontaneously-beating isolated right atria of both rats and guinea pigs, alone and in the presence of drugs that enhance the force of contraction (inotropy) of cardiac muscle. Our intent was two-fold: a) to re-examine aspects of the cardiac response described by Apstein (1978) that appeared to suggest that PGB_x accentuates the actions of the β-adrenergic agonist isoproterenol in isolated ischemic rabbit hearts during the post-ischemic recovery period and, based on this response, b) to develop an organ level biological assay that would serve as an alternative to the mitochondrial assay and that could more closely reflect activities that are therapeutically relevant.

Initial studies were performed on isolated rat right atrial preparations. Right atria were surgically removed from male Sprague Dawley derived albino rats of both sexes [Hap:(SD)BR, 200-500 gm] and suspended under 0.5 gm tension in a 10 ml tissue bath containing Kreb's solution aerated with 95% oxygen-5% carbon dioxide. Temperature was maintained at 36 ± 0.1°C. Rate (chronotropy) and force (inotropy) were monitored with either Grass or Narco Biosystems oscillographs equipped with isometric transducers.

Relatively high concentrations of PGB_x (e.g. 100 µg/ml bath) exert a mild increase in contractility (12 to 15% max), an effect which is not seen, or only occasionally seen, at lower concentrations. No rate changes were observed. When these tissues were preincubated, with varying concentrations of PGB_x and subsequently challenged with a beta stimulant, such as (-)-isoproterenol, a striking increase in the force of contraction was observed.

The largest increase in contractility occurred at the "top" of the dose-response curve (i.e. when isoproterenol concentration was high), suggesting that PGB_x increases the apparent intrinsic activity of isoproterenol. This activity was related to the concentration of PGB_x (8-12 µg/ml bath produced significant and, what appeared to be, maximum effects).

Histamine, a drug whose inotropic action is initiated by a mechanism different from isoproterenol, also produced an increased force of contraction that is intensified by PGB_x. (Histamine does not produce a positive inotropic action in rats so only guinea pigs were used for this study). Although there is some quantitative difference between the effects of PGB_x on isoproterenol and histamine, the results are qualitatively similar and strongly suggest that PGB_x operates by a mechanism that is independent of adrenergic or histaminergic membrane receptors.

The contractility-enhancing actions of PGB_x provided the basis for an organ bioassay for the material and additional studies were undertaken to establish assay validity and to determine the protocol necessary to maximize assay reliability.

Although it was possible to use the enhancement of either (-)-isoproterenol or histamine as the basis for the organ assay, we chose the isoproterenol system solely because we have a somewhat better understanding of its dynamics.

Fig. 1 illustrates the type of effect PGB_x exerts on guinea pig right atrial contractility in the presence of (-)-isoproterenol. In this example, tissues were incubated with PGB_x (8 µg/ml bath) 3 min prior to the first addition of isoproterenol. The contractile agonist was introduced in graded increments until a maximum inotropic response is seen. This method of constructing cumulative dose-response curves was originally described by

Van Rossum and van der Brink in 1963. Note that the DR curves for isoproterenol generated before PGB_x treatment and 20 minutes after washing the tissue free of PGB_x are virtually identical.

Incubating the tissues with PGB_x for periods less than 3 or greater than 10 minutes (prior to isoproterenol addition) produce less intense PGB_x responses.

Briefly stated, the bioassay is based upon the ability of PGB_x to increase the contractility-provoking effect of a fixed concentration of (-)-isoproterenol and the conditions of the assay are summarized in Table I. The assay is a "relative potency" assay and all PGB_x preparations, analogues and fractions are compared to the same reference standard PGB_x (currently we use Fraction 2, preparation 28).*

Fig. 2 portrays the log dose-response relationship of PGB_x. Although the line was constructed from data covering a dose range of 1 through 16 µg/ml, the useable dose-responsive range of PGB_x appears to be somewhat narrower, in the neighborhood of 2-12 µg/ml bath. This is extremely narrow, and the bioassayist must subject all unknown compounds to preliminary dosing studies in order to estimate the concentrations that will fall within a dose-responsive range. On the other hand, the steepness of the dose-response curve makes it easier to discriminate between compounds having different potencies. The slope of the curve depicted in Fig. 2 is 38.5 percentage units/log dose unit, the correlation coefficient is 0.79, and the index of precision (lambda) is 0.258.

The bioassay protocol that has been developed appears to be reasonably reliable. Since PGB_x effect is rapidly terminated following replacement of the bathing solution and since the atrial tissue recovers completely from

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*One of us (DDM) is now preparing a large scale sample which will be standardized against preparation 28 and subsequently assume the role of a "house standard".

the effects of PGB_x after equilibration with fresh medium for approximately 20 minutes, it is possible to use the same heart for repeated PGB_x trials. The one cautionary note that must be included here is that some hearts are inhibited by PGB_x and these should not be used in the assay. This selective exclusion process clearly prevents us from extrapolating our potency assessments to the heart population generally. However, it does allow us to make a valid relative potency determination for those PGB_x samples that exhibit some degree of positive inotropic behavior. In other words, the exclusion process does not diminish the assay's validity.

A Latin square design requiring 2 concentrations of reference standard PGB_x and 2 concentrations of the compound of unknown potency (all falling within the dose-responsive range) has been adopted. Each heart is exposed to all 4 doses of PGB_x (each of which is challenged by 2×10^{-8}M of (-)-isoproterenol). The minimum amount of data necessary to make a potency evaluation requires 4 hearts (Table 2). A more reliable estimate of potency can be made by expanding the protocol to include 12 hearts (3 per dosing sequence). Obviously, limitations in the amount of compound available for evaluation will influence the type of protocol that can be used. In our opinion the 4 heart design provides an adequate assessment of potency with an acceptable degree of reliability. This conclusion is justified because of the results of assays such as the one depicted in Fig. 3. In this experiment the reference standard PGB_x solution was diluted with an equal volume of saline. This preparation, which had a known potency which was one-half that of the authentic reference standard, was assayed against the authentic RS. The potency of this preparation was estimated to be 0.41 relative to the RS with 95% confidence limits of 0.30-0.56. *

*An abstract of a paper describing the assay (which will be presented at the Eighth International Congress of Pharmacology in Tokyo) is included in Appendix A.

Effect of PGB_x on the vasopressor response to segmental stimulation of sympathetic outflow in adrenalectomized, pithed rats. In the course of a multidimensional activity screen, designed to examine the effects of PGB_x on systems responsive to known prostaglandins, we observed that PGB_x produced a significant inhibition of vascular muscle tone induced by electrical stimulation of innervating sympathetic nerves. Sixty male Wistar rats [Hap:(WI)BR, 210-365 gm] prepared according to the method of Gillespie and Muir (1967) were used. Rats which had been atropinized were anesthetized with ether, pithed and artificially ventilated. A stimulating electrode was introduced into the vertebral canal via the right orbit and an indifferent subcutaneous electrode attached through the dorsal skin surface. The stimulated region was at the level of T_9-L_1. Tubucurarine, IV, was administered and submaximal 1 msec, 20 volt pulses were delivered at graded frequencies for 14 sec periods at 2 min intervals. Systolic blood pressure was directly measured from the femoral artery (Statham P 23 10 transducer) and drugs were administered through a catheter inserted into the femoral vein (Fig. 4). Both adrenalectomized and nonadrenalectomized rats were used.

The stimulus frequency-vasopressor response profile in adrenal ablated rats differs markedly from that seen in nonablated animals due to the massive release of catecholamine from the adrenals of the latter group (Fig. 5). In these acute experiments, PGB_x as well as PGB_1 and PGE_1 were examined. Fig. 6 depicts the effects of PGB_x on the frequency-vasopressor response at various time periods following drug administration. The data were corrected for changes in the control that are associated with deterioration of the preparation with time. Figs. 7 and 8 portray similar curves for PGB_1 and PGE_1, respectively. Although the experiments are limited to single doses of each com-

pound, differences in the responses to these drugs can be noted. The effects of a range of PGB_x doses including 5, 2.5, 1 and 0.5 mg/kg are now in progress.

In several chronic experiments PGB_x, 1.2 and 6 mg/kg, was injected SC twice a day for seven days in rats that were subsequently prepared according to the method of Gillespie and Muir (1967). Control rats received phosphate buffer vehicle in place of PGB_x. Frequency-pressor response curves were generated for adrenal ablated animals (Fig. 9).

PGB_x, in single bolus IV injections of 10 mg/kg, inhibited the stimulus frequency-pressor response to electrical stimulation of sympathetic outflow from the spinal cord in adrenal ablated rats. The inhibition exerted by PGB_x was slow in onset and progressed with time. Following SC injection of PGB_x for seven days, the frequency-pressor response to electrical stimulation decreased markedly and in a dose-dependent fashion. PGE_1 produced a relatively short-lasting inhibition of the frequency-pressor response to electrical stimulation. On the other hand, PGB_1 (10 mg/kg IV) caused a significant augmentation of the pressor response to electrical stimulation in both adrenalectomized and pithed rats. PGB_x (and PGE_1) could exert their actions by stimulating or sensitizing vascular beta receptors (which promote vasodilatation) and in order to examine this hypothesis PGB_x, PGB_1 and PGE_1 were examined for their ability to produce smooth muscle relation in a beta receptor-bearing tissue (guinea pig trachea) using the method of Castillo and DeBeer (1947). Fig. 10 reveals that unlike PGB_1 and PGE_1, PGB_x produced no significant relaxation of tracheal muscle. It is now possible to consider alpha adrenergic blockade and interference with sympathetic nerve function as alternative hypotheses to explain the impairment of vascular response to neural stimulation.

If we examine the vascular response of the adrenalectomized pithed rat to norepinephrine injection (instead of electrical stimulation of sympathetic outflow) we find that the effects of PGB_x are unimpressive (Fig. 11). Thus, it seems unlikely that we are witnessing an effect related to alpha adrenergic blockade.

The influence of PGB_x and PGE_1 on heart rate and blood pressure in conscious unrestrained rats. Following the method of Weeks and Jones (1960), a polyethylene catheter was implanted in the abdominal aorta and exteriorized 6-24 hours prior to the experiment. Blood pressure and heart rate were monitored through this catheter. Intravenous injections were made through a catheter inserted into the left external jugular vein (Fig. 12). PGB_x (1, 3 and 10 mg/kg) and PGE_1 (0.01, 0.03, 0.1 and 0.3 mg/kg) were injected at 10 or 20 min intervals (Figs. 13 and 14). Doses of 1-10 mg/kg of PGB_x, IV, had no significant effect on basal heart rate or blood pressure, while PGE_1 produced both tachycardia and reduction in blood pressure. The tachycardia could be prevented by premedicating the animal with the beta-receptor antagonist, propranolol (1 mg/kg), but this drug could not block the vasodilating action of PGE_1. The vascular response to PGE_1 cannot, therefore, be readily attributed to beta receptor stimulation. It has been suggested by others (e.g. Baum and Shropshire, 1971) that prostaglandins of the E series are able to inhibit sympathetic transmission. Although they are quite different in many respects, PGB_x and PGE_1 may produce their vasodilator actions by a similar mechanism. PGB_x which exerts little or no direct cardioacceleratory action and no bronchodilator effect could represent a new and novel class of potentially useful antihypertensive compounds. Incidentally, if PGB_x exerts an inhibitory effect on sympathetic outflow or transmitter release from sympathetic nerve terminals, as we suggest, this may explain the observation by

Moss (1979) that the "shock lung" syndrome provoked by cerebral hypoxia can be prevented by lung denervation or PGB_x.*

Influence of PGB_x on functions of the ischemic heart. Elaborating on a technique originally described by Endoh and Hashimoto (1970), we are able to simultaneously examine the effects of drugs and on some of the electrical and mechanical functions of portions of the canine heart. The method involves the surgical removal of papillary muscle, a segment of the right atrial appendage (in which is embedded the SA node), and AV nodal tissue from a recipient dog. The tissues are perfused through an accessible artery with blood from a donor or support dog (Fig. 15B). The isolated tissues are surrounded by a water jacket warmed to 37-38°C. Perfusion is at constant pressure (100 mmHg) and blood flowing from the preparation (and excess blood passing through the pneumatic resistance unit) are collected in a reservoir and returned to the support dog via the right external jugular vein. The cardiac tissue is sutured to a plastic plate. Papillary muscle is driven by bipolar electrodes sutured to the interventricular septal surface (Fig. 15A). Similar electrodes attached to the right atrial and AV preparations monitor the electrocardiogram. Developed tension in all three types of tissues is measured isometrically with a strain gauge transducer. We have recently completed a characterization of the effects of arterial occlusion on the loss of various parameters of cardiac function and are now completing experiments designed to examine the effect of various doses of PGB_x on those functions. Figs. 16 and 17 portray

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*Selected portions of these data were presented at the Fall Meeting of the American Society of Pharmacology and Experimental Therapeutics, Rochester, Minn., Aug. 20, 1980. The abstract is reproduced in the Appendix B.

the effects of total occlusion of the afferent artery on the functional behavior of 3 types of isolated cardiac tissue, viz., SA node bearing atrium, AV node bearing septal, and Purkinje bearing papillary muscle. Thirty minute occlusion produced impairment of both automaticity and contractility in all tissues although the degree of disfunction varied. Papillary muscle was most dramatically altered with regard to both functions. Reestablishing blood flow results in a rapid restoration of functionality although force-frequency studies currently in progress clearly suggest that the tissues are no longer able to respond to higher frequency stimulation (> 2 Hz) and have lost much of their functional reserve.

Pretreatment of these tissues with varying doses of PGB_x 15 min prior to occlusion reduces the consequences of ischemia. Fig. 18 illustrates the influence of prophylactic PGB_x (administered to the support dog in doses of 0.1-2.5 mg/kg on the contractility of electrically paced papillary muscle. Lower doses (0.1 and 0.5 mg/kg) significantly prolonged the functional survival time of the muscle (from 10 min post occlusion to at least 30 min). These studies are not as yet complete but it appears that PGB_x is able to exert a significant effect on the ischemic syndrome. Not only is there a less dramatic loss of cardiac functionality following arterial occlusion but the force-frequency curves of oxygen deprived tissues more clearly resemble those of normal tissue.*

*Selected portions of these data will be presented at the meeting of the Federation of American Societies for Experimental Biology, Atlanta, GA. Abstracts of the 2 presentations are included in Appendices C and D.

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Table I. Bioassay Conditions

1. Variable measured — enhancement of drug-induced contractility in isolated spontaneously-beating guinea pig right atria.

2. Animal — English short hair guinea pig, 300-500 grams, both sexes.

3. Incubation chamber — 10 ml

4. Medium — Kreb's solution (an isotonic, bicarbonate buffered electrolyte solution compatible with tissue survival)

5. Aeration — 95% oxygen, 5% carbon dioxide

6. Chamber temperature — 36 ± 0.05°C

7. Diastolic tension — 500 mg

8. Transducer — isometric force displacement type

9. Agonist — (-)-isoproterenol, 2×10^{-8} M

10. PGB_x incubation time — 3-5 minutes

11. Design — Four point (2x2) parallel line assay with graded response and 4 replications for each dose.

Table II. Latin square dosing protocol for the PGB_x organ assay. Box numbers indicate the way in which doses have been assigned to each of 4 hearts.

Heart	Dosing Sequence 1	2	3	4
A	1[a]	2	3	4
B	2	3	4	1
C	3	4	1	2
D	4	1	2	3

[a] 1 and 3 are low and high doses of the reference standard; 2 and 4 are low and high doses of the test preparation.

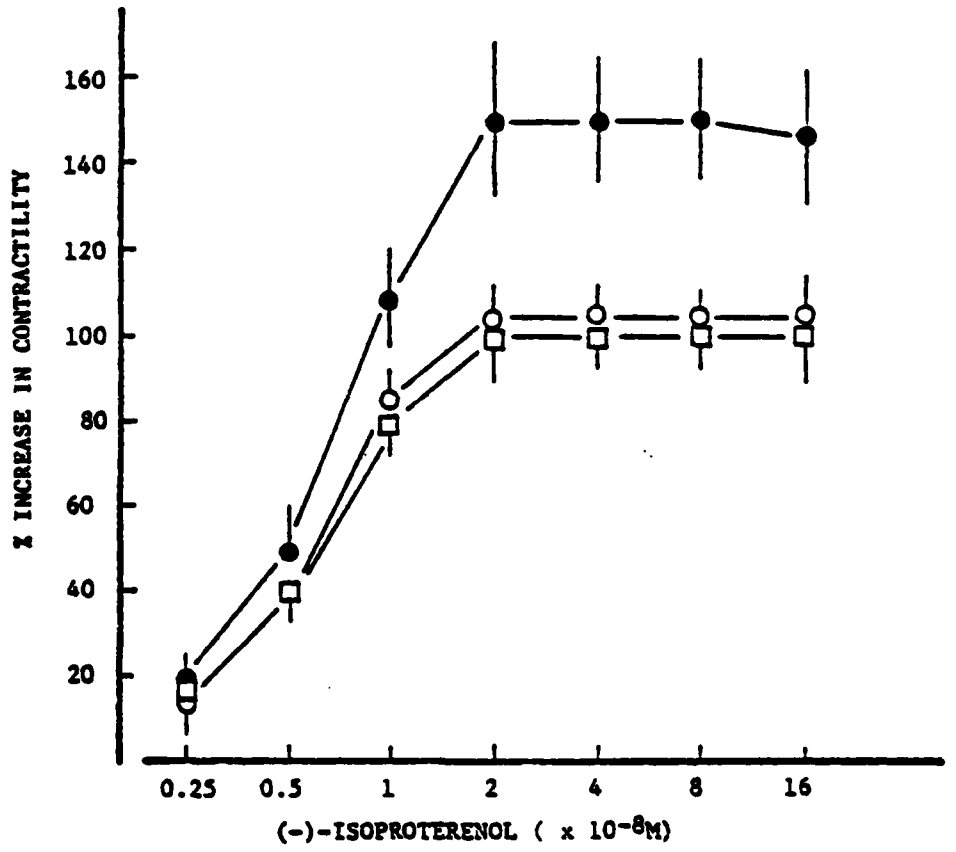


Fig. 1 Log dose-response curves for (-)-isoproterenol in the presence and absence of PGB_x. Spontaneously beating guinea pig right atria were incubated for 3 min with PGB_x before induction of the agonist. ⊏ Agonist alone in the absence of PGB_x; ● PGB_x, 8 µg/ml bath; ○ agonist alone, 20 min after PGB_x washout (N = 6).

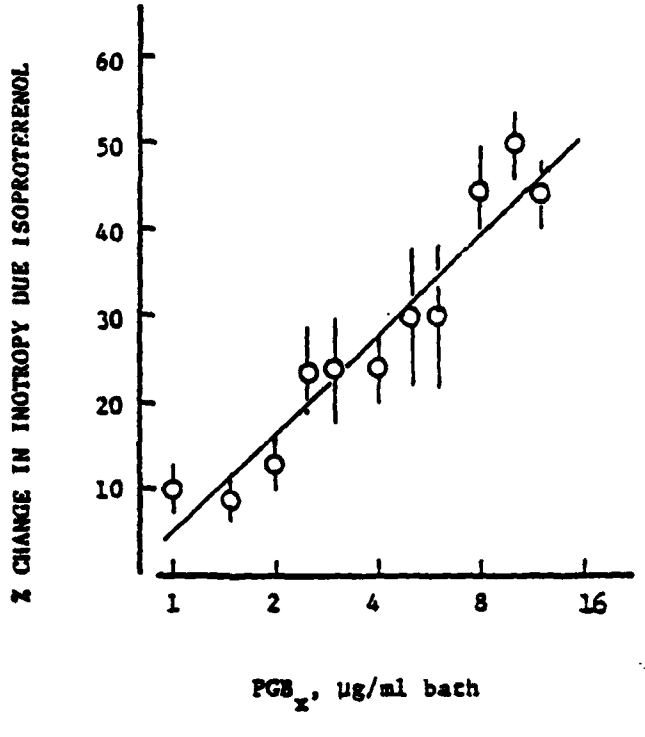


Fig. 2 Log dose-response curve for PGB_x on inotropic response guinea pig right atria to (-)-isoproterenol (2×10^{-8}M). Four hearts contributed to each datum point. Slope is 38.53 ± 5.06 percentage units/log dose unit with 95% confidence limits of 29.7-47.4; r = 0.79; λ = 0.258.

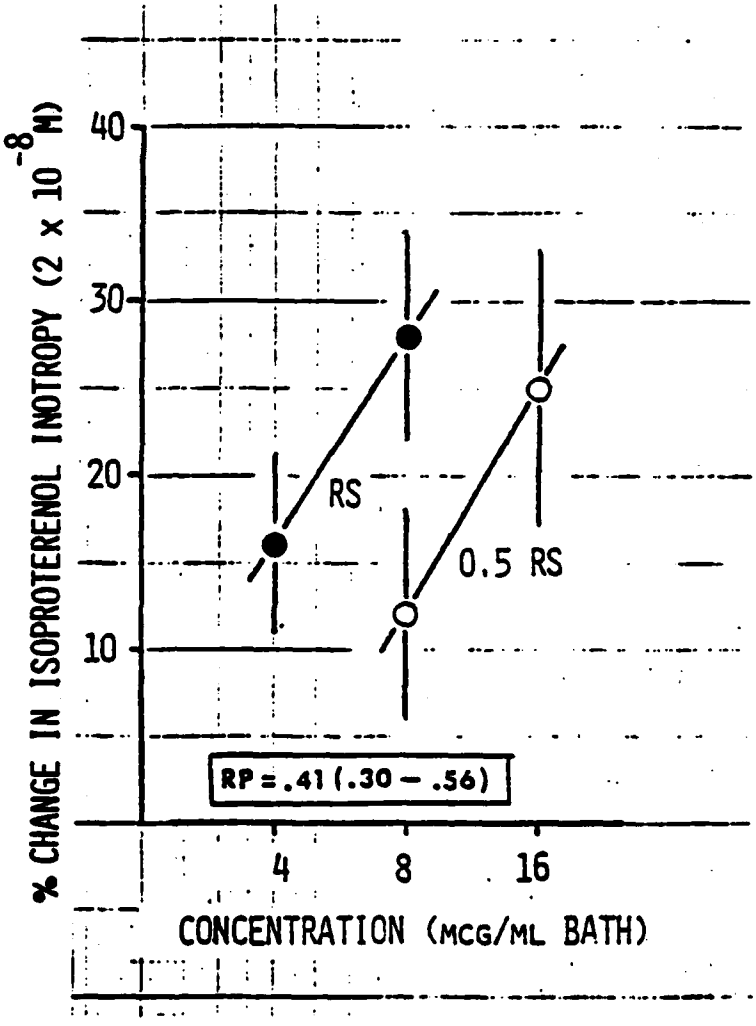


Fig. 3. Relative potency assay of PGB_x. The test sample (0.5 RS) consisted of the reference standard (RS) material diluted to one-half the original potency. N=4/point.

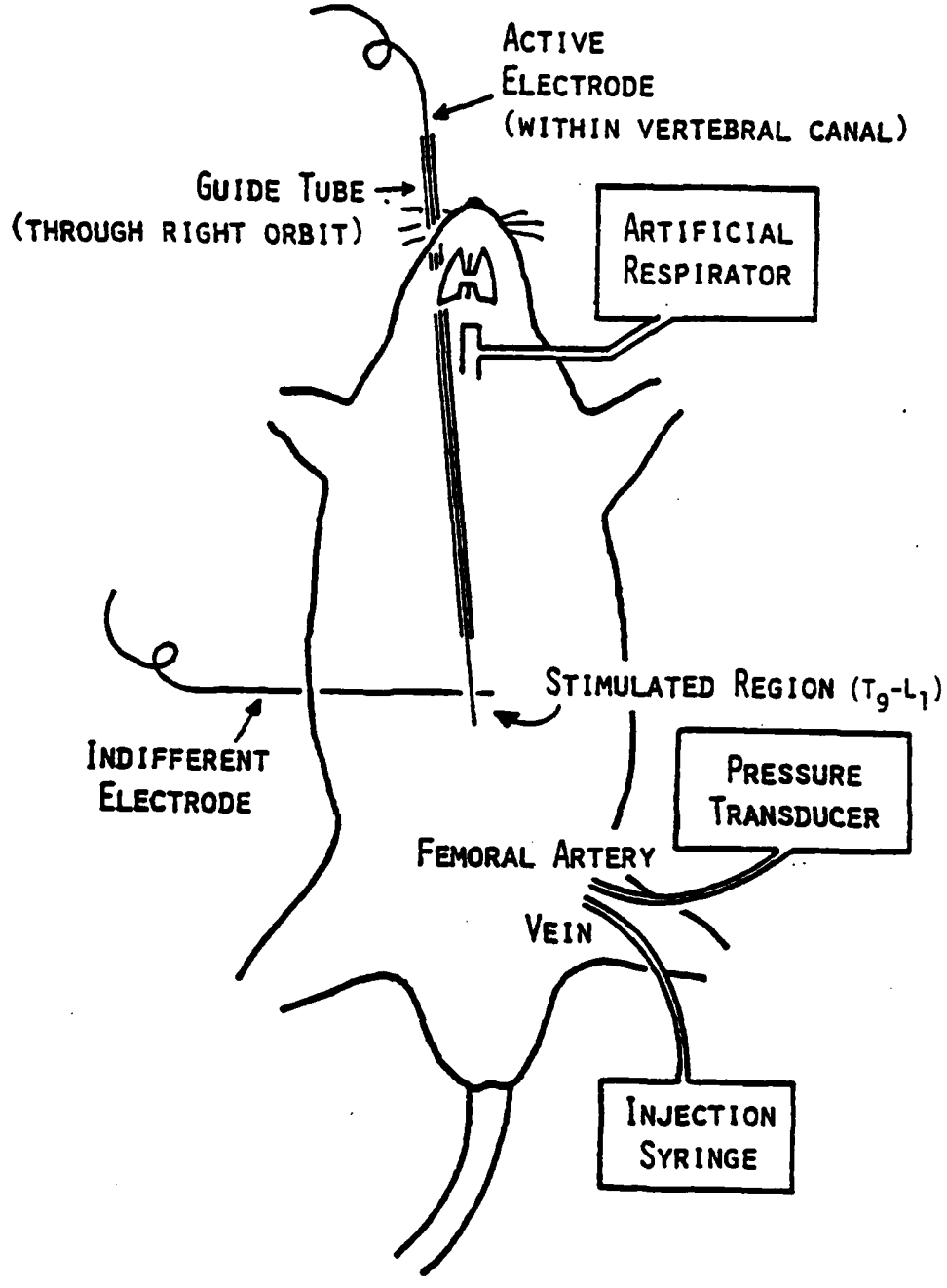


Fig. 4. Arterial blood pressure and heart rate are monitored during electrical stimulation of sympathetic motor neurons that innervate vascular smooth muscle. Stimulus frequency-vasoconstrictor response curves can be constructed in the presence and absence of PGB_x. The animal is pithed and artificially ventilated.

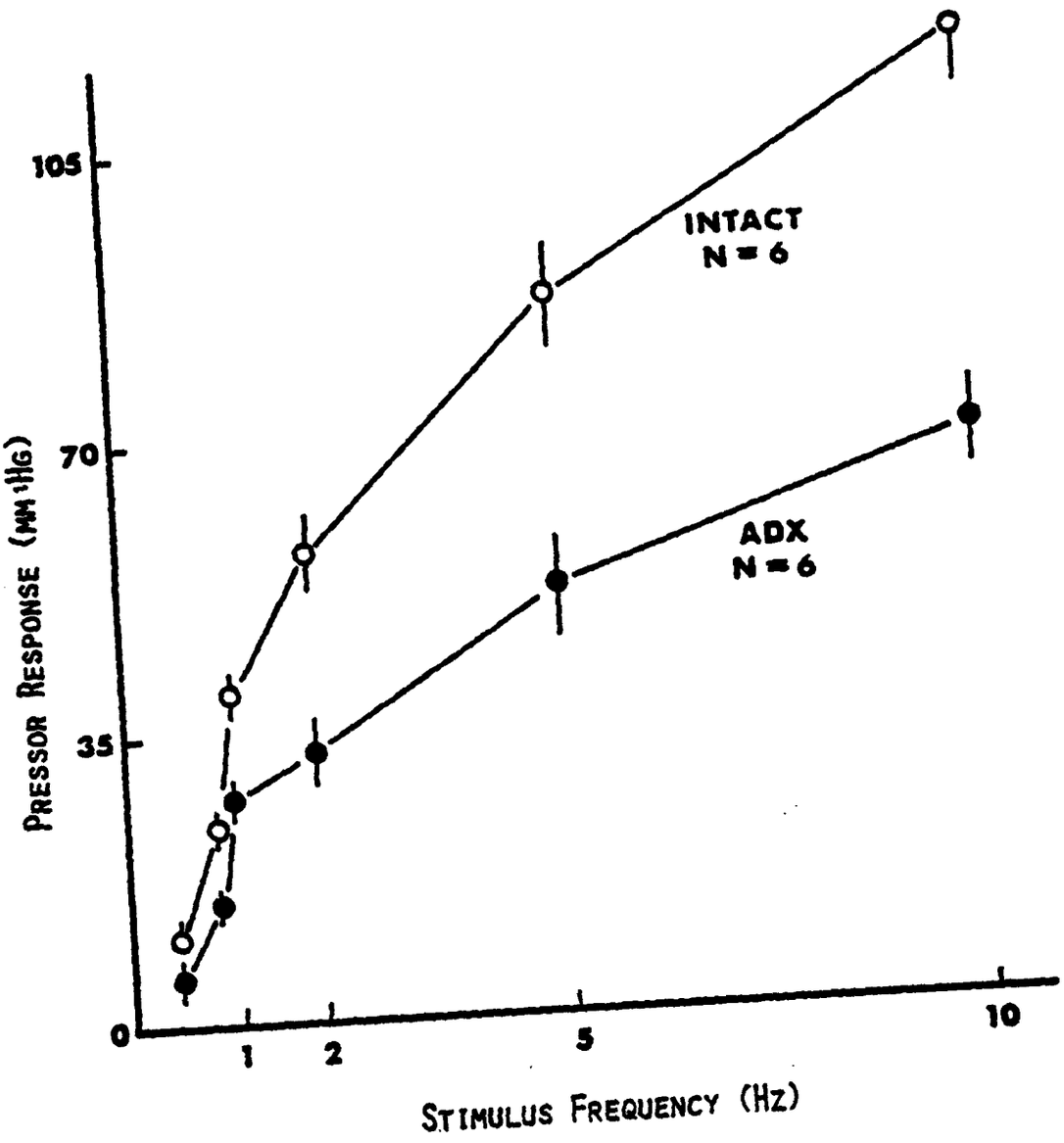


FIG. 5 RELATIONSHIP BETWEEN PRESSOR RESPONSE (CHANGE IN SYSTOLIC BLOOD PRESSURE FROM PRESTIMULUS BASELINE) AND FREQUENCY OF ELECTRICAL STIMULATION OF SYMPATHETIC VASOMOTOR FIBERS IN ADRENALECTOMIZED, PITHED RATS (ADX) AND NON-ADRENALECTOMIZED, PITHED RATS (INTACT).

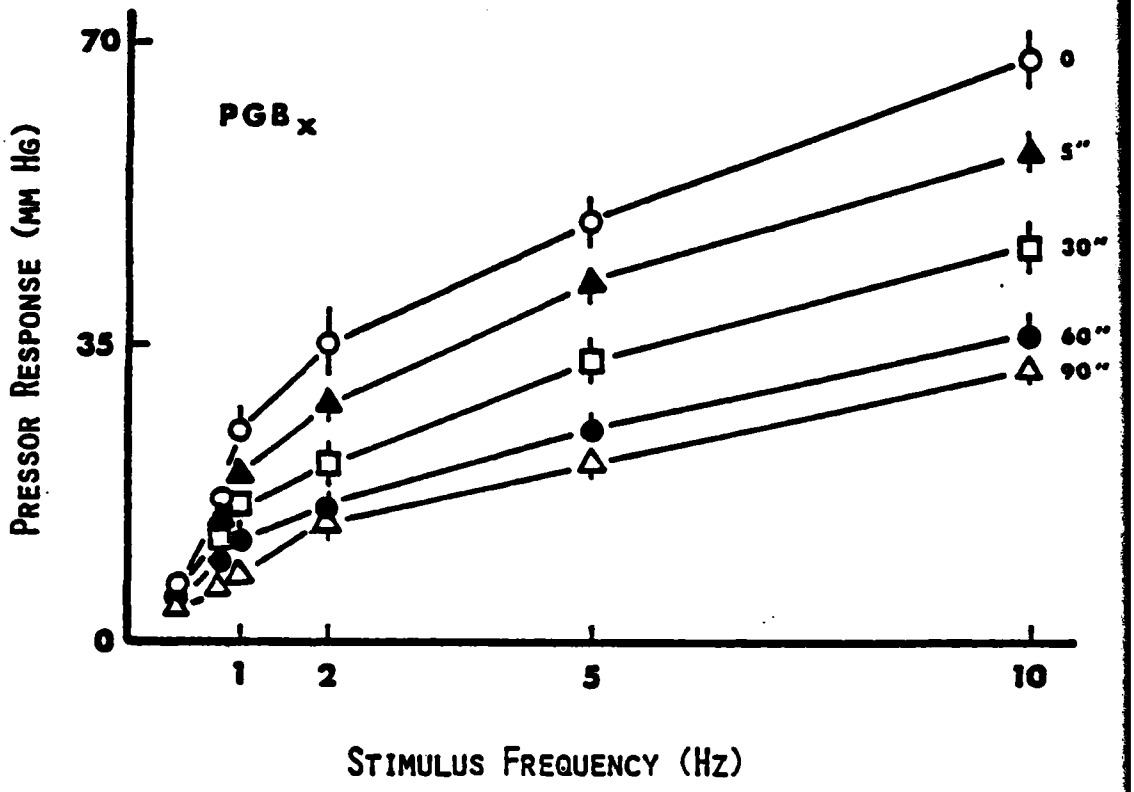


FIG. 6 INFLUENCE OF PGB_X (10 MG/KG, IV BOLUS) ON PRESSOR RESPONSE TO ELECTRICAL STIMULATION OF SYMPATHETIC VASOMOTOR FIBERS IN ADRENALECTOMIZED, PITHED RATS. EACH CURVE REPRESENTS A STIMULUS-RESPONSE PROFILE AT A DESIGNATED TIME AFTER INJECTION (N = 6).

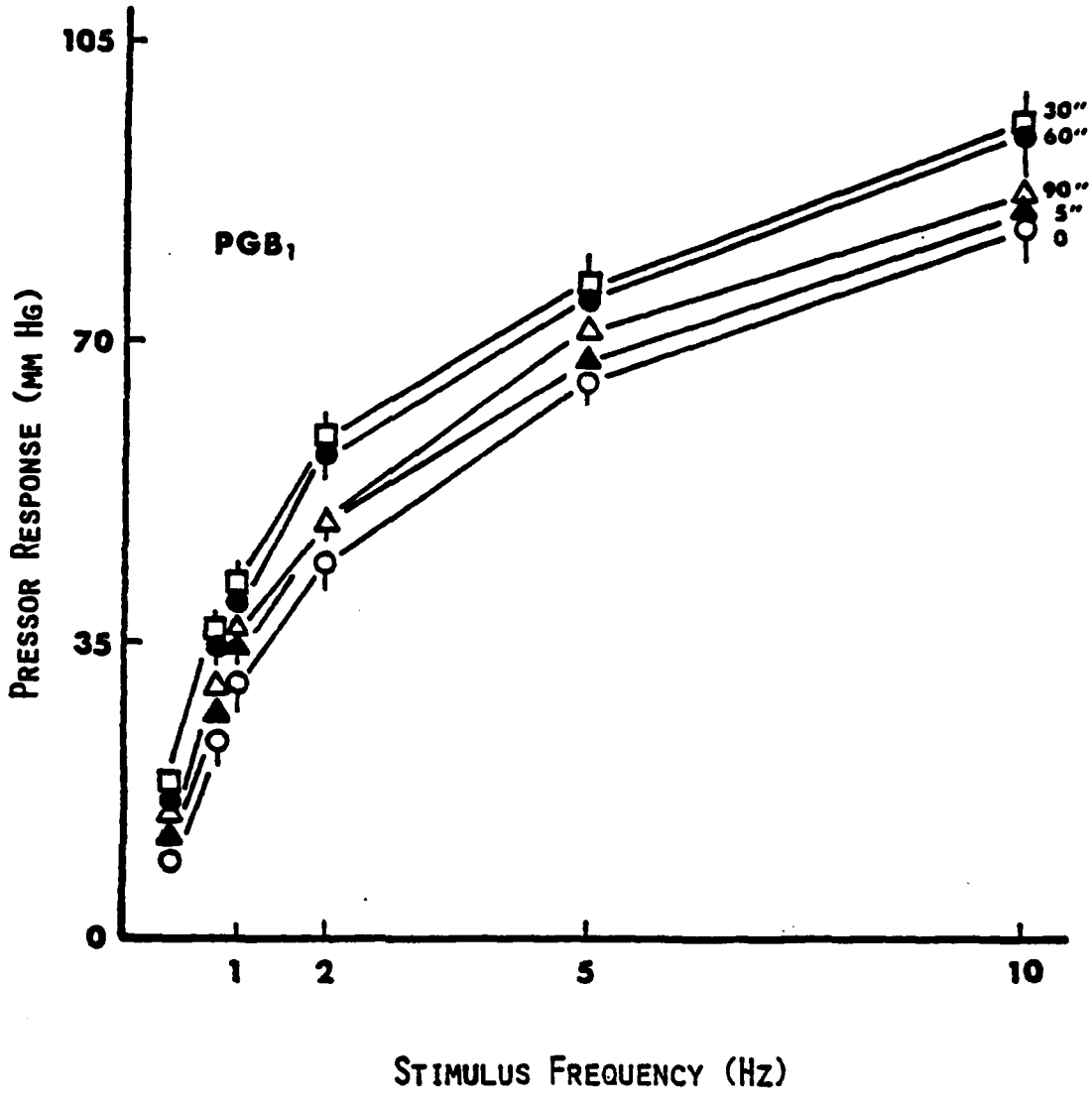


FIG. 7 . INFLUENCE OF PGB_1 (10 MG/KG, IV BOLUS) ON PRESSOR RESPONSE TO ELECTRICAL STIMULATION OF SYMPATHETIC VASOMOTOR FIBERS IN ADRENALECTOMIZED, PITHED RATS. EACH CURVE REPRESENTS A STIMULUS-RESPONSE PROFILE AT A DESIGNATED TIME AFTER INJECTION (N = 5).

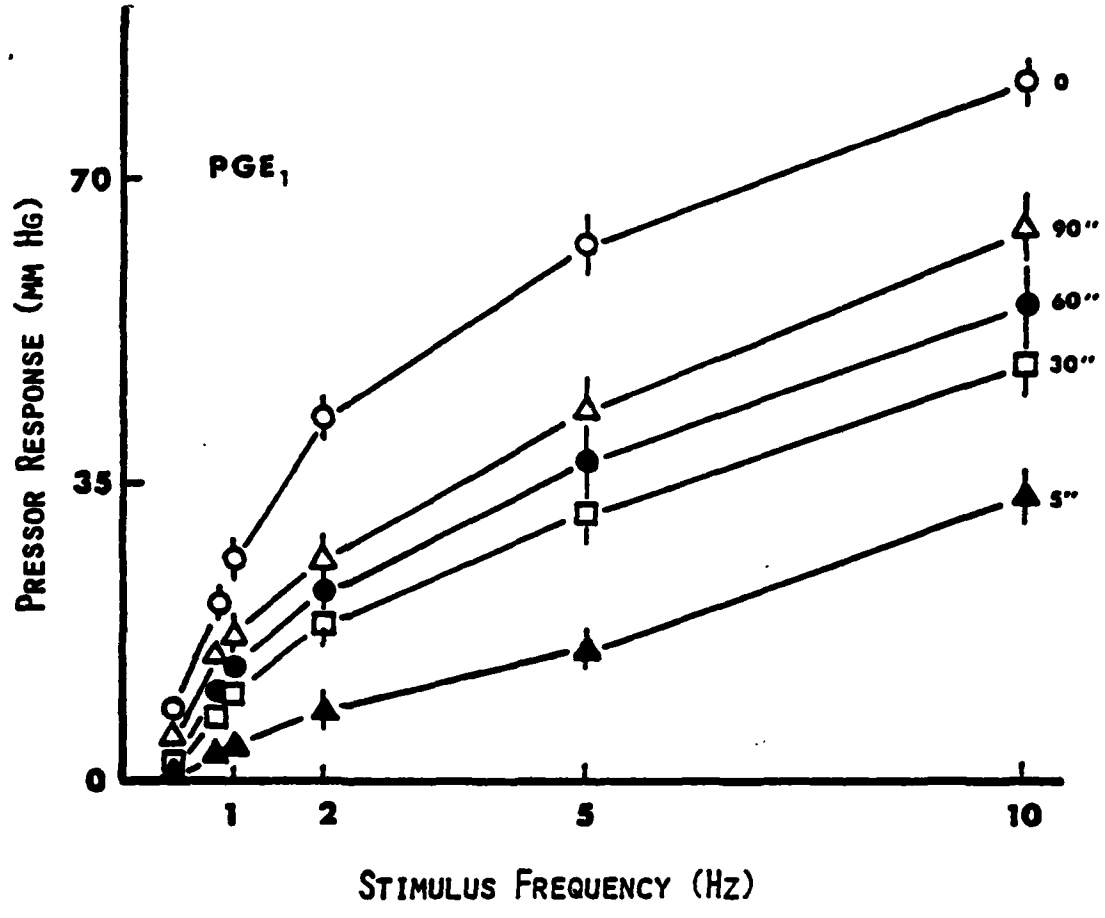


FIG. 8 INFLUENCE OF PGE_1 (1 MG/KG, IV BOLUS) ON PRESSOR RESPONSE TO ELECTRICAL STIMULATION OF SYMPATHETIC VASOMOTOR FIBERS IN ADRENALECTOMIZED, PITHED RATS. EACH CURVE REPRESENTS A STIMULUS-RESPONSE PROFILE AT A DESIGNATED TIME AFTER INJECTION (N = 5).

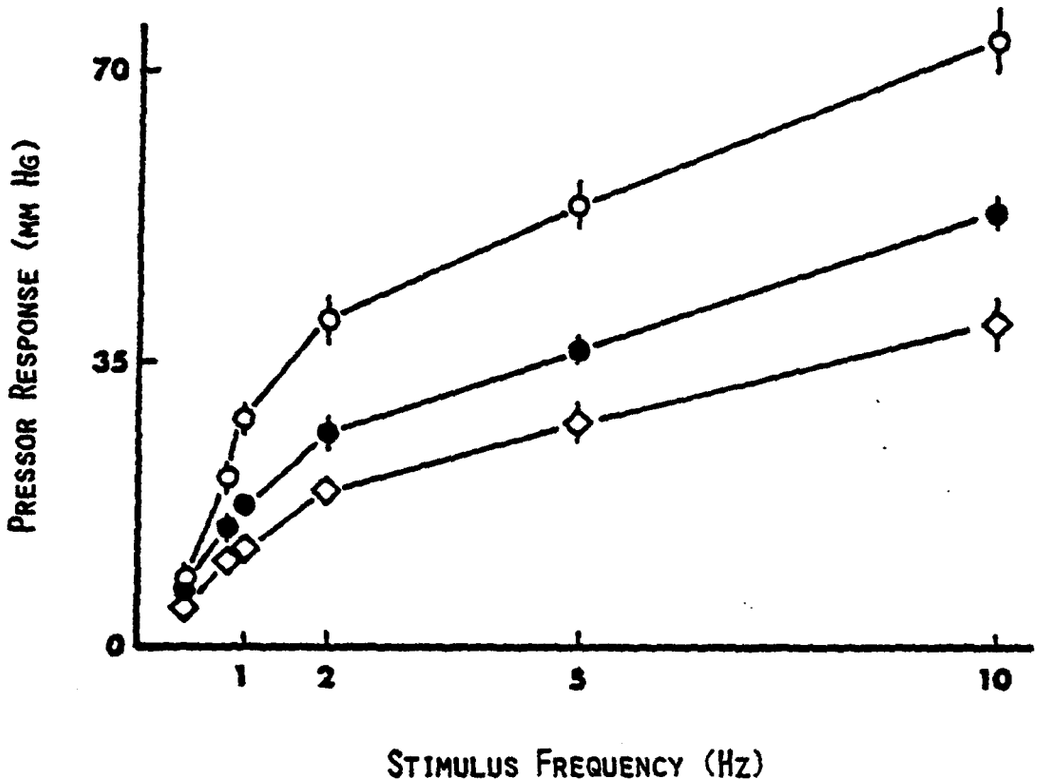


FIG. 9 INFLUENCE OF CHRONIC PGB_X PRETREATMENT ON PRESSOR RESPONSE TO ELECTRICAL STIMULATION OF SYMPATHETIC VASOMOTOR FIBERS IN ADRENALECTOMIZED, PITHED RATS. ○ CONTROLS (N = 7); ● PGB_X, 2 x 1.2 MG/KG/DAY FOR 7 DAYS (N = 5); ◇ PGB_X, 2 x 6 MG/KG/DAY FOR 7 DAYS (N = 8).

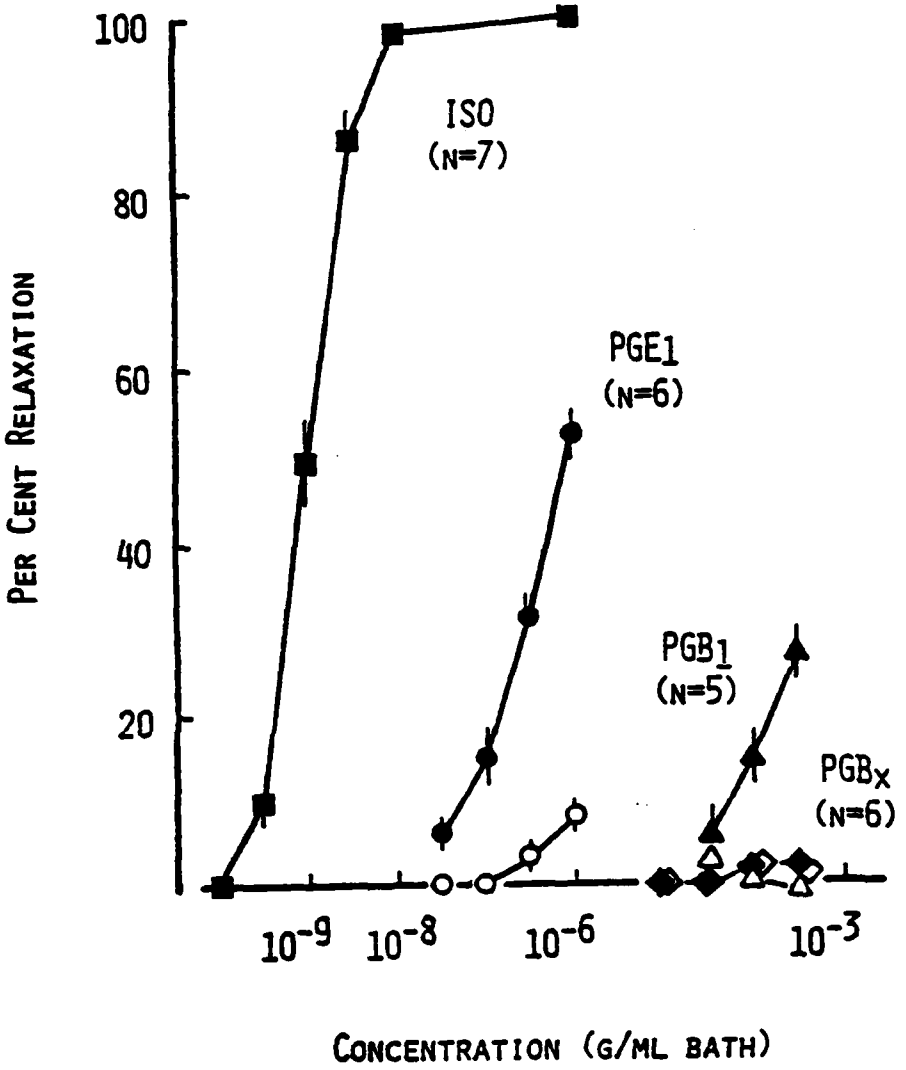


FIG. 10 EFFECTS OF ISOPROTERENOL (■), PGE_1 (●), PGE_1 VEHICLE (○), PGB_1 (▲), PGB_1 VEHICLE (△) PGB_X (◆), AND PGB_X VEHICLE (◇) ON GUINEA PIG TRACHEAL CHAIN PREPARATION IN THE PRESENCE OF HISTAMINE (3×10^{-6} G/ML).

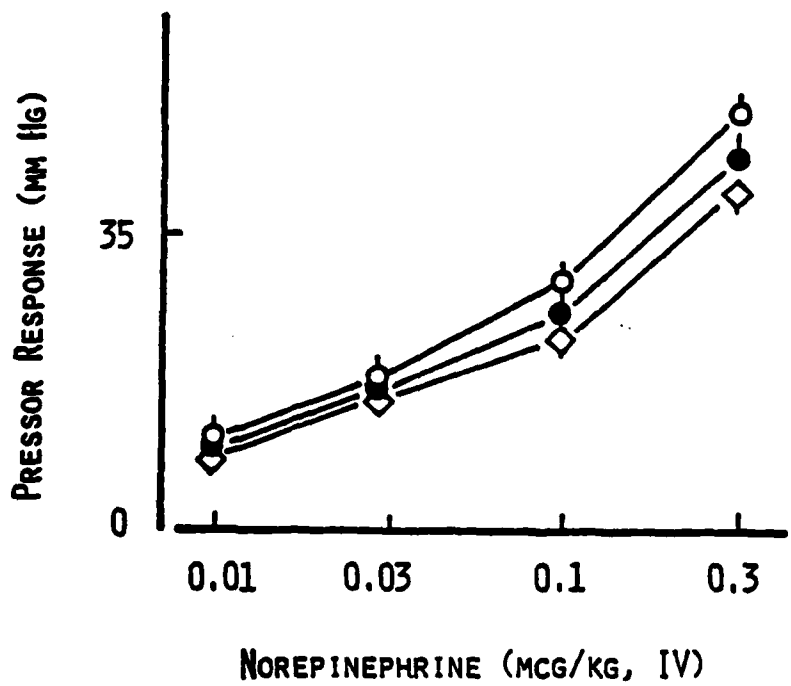


FIG. 11 INFLUENCE OF CHRONIC PGB_X PRETREATMENT ON PRESSOR RESPONSE TO NOREPINEPHRINE IN ADRENALECTOMIZED, PITHED RATS. ○ CONTROL (N = 7); ● PGB_X, 2 x 1.2 MG/KG/DAY FOR 7 DAYS (N = 5); ◇ PGB_X, 2 x 6 MG/KG/DAY FOR 7 DAYS (N = 8).

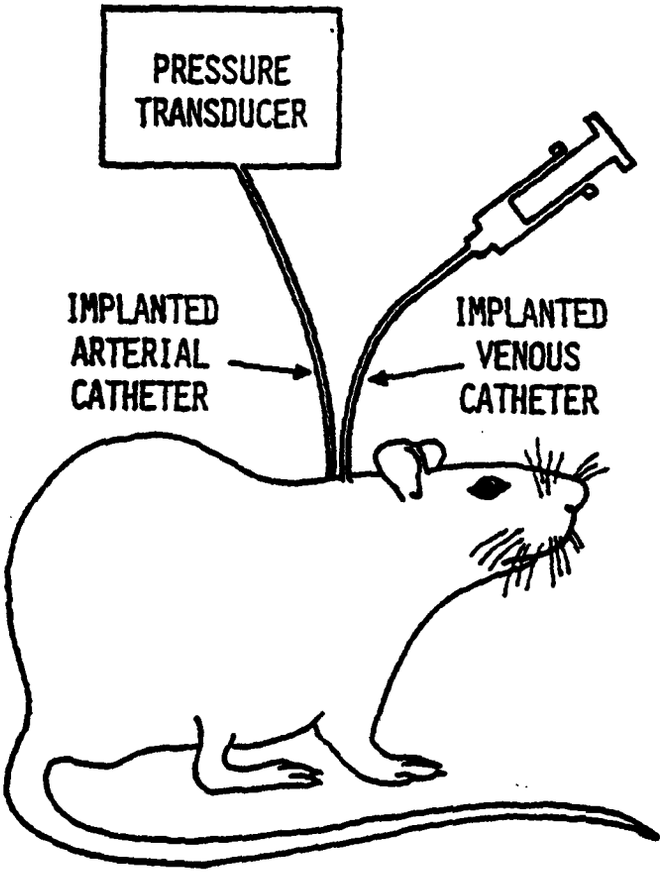


Fig. 12 Two exteriorized catheters inserted into the jugular vein and abdominal aorta facilitate the IV administration of PGB_x and the monitoring of BP and heart rate in conscious, unrestrained animals.

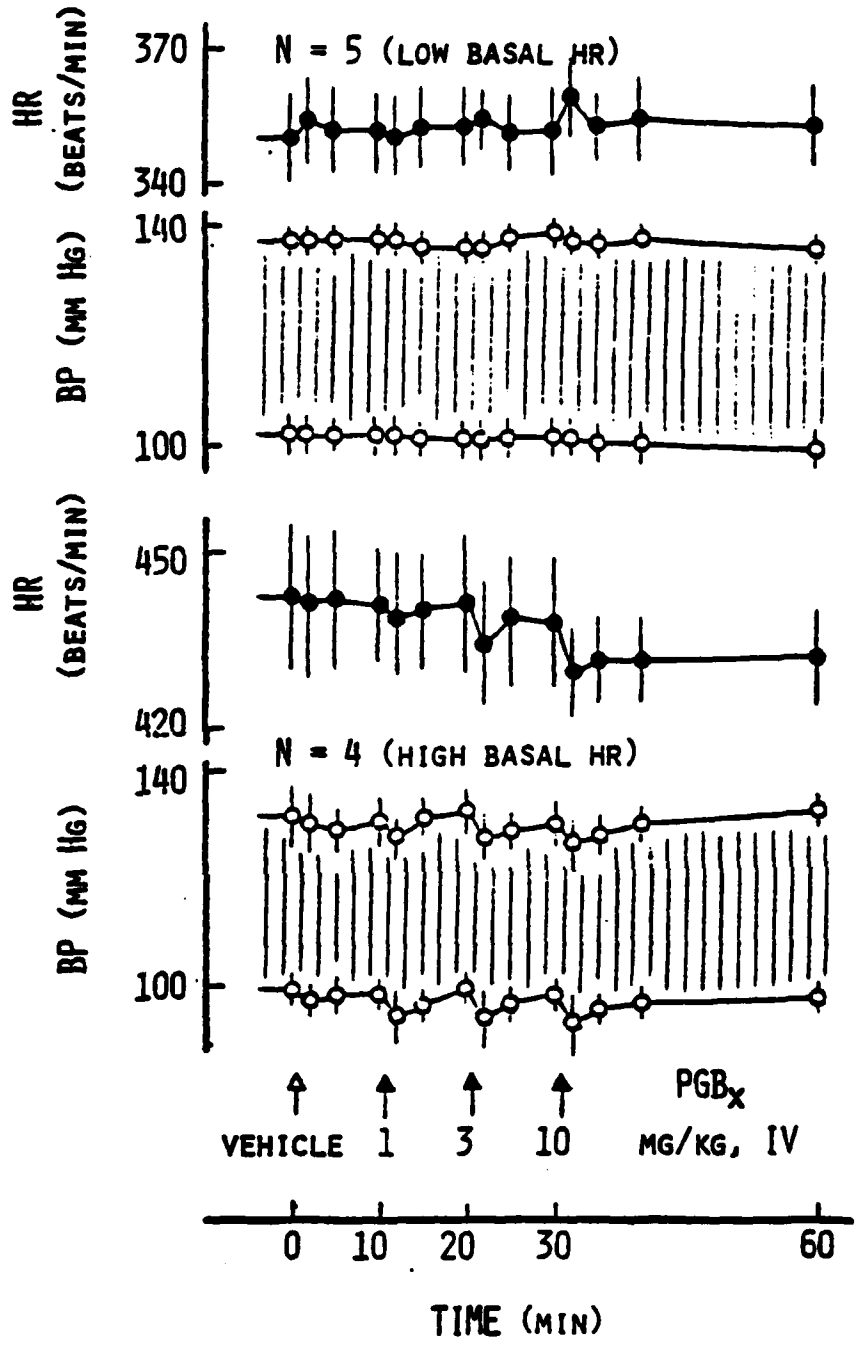


Fig. 13 Influence of PGB_x (1-10 mg/kg, IV) on heart rate and arterial blood pressure of conscious, unrestrained rats. Animals were partitioned into 2 categories: those having low basal HR (top pair of recordings) and those having high basal HR (lower pair or recordings). Neither group exhibited significant changes in the presence of PGB_x.

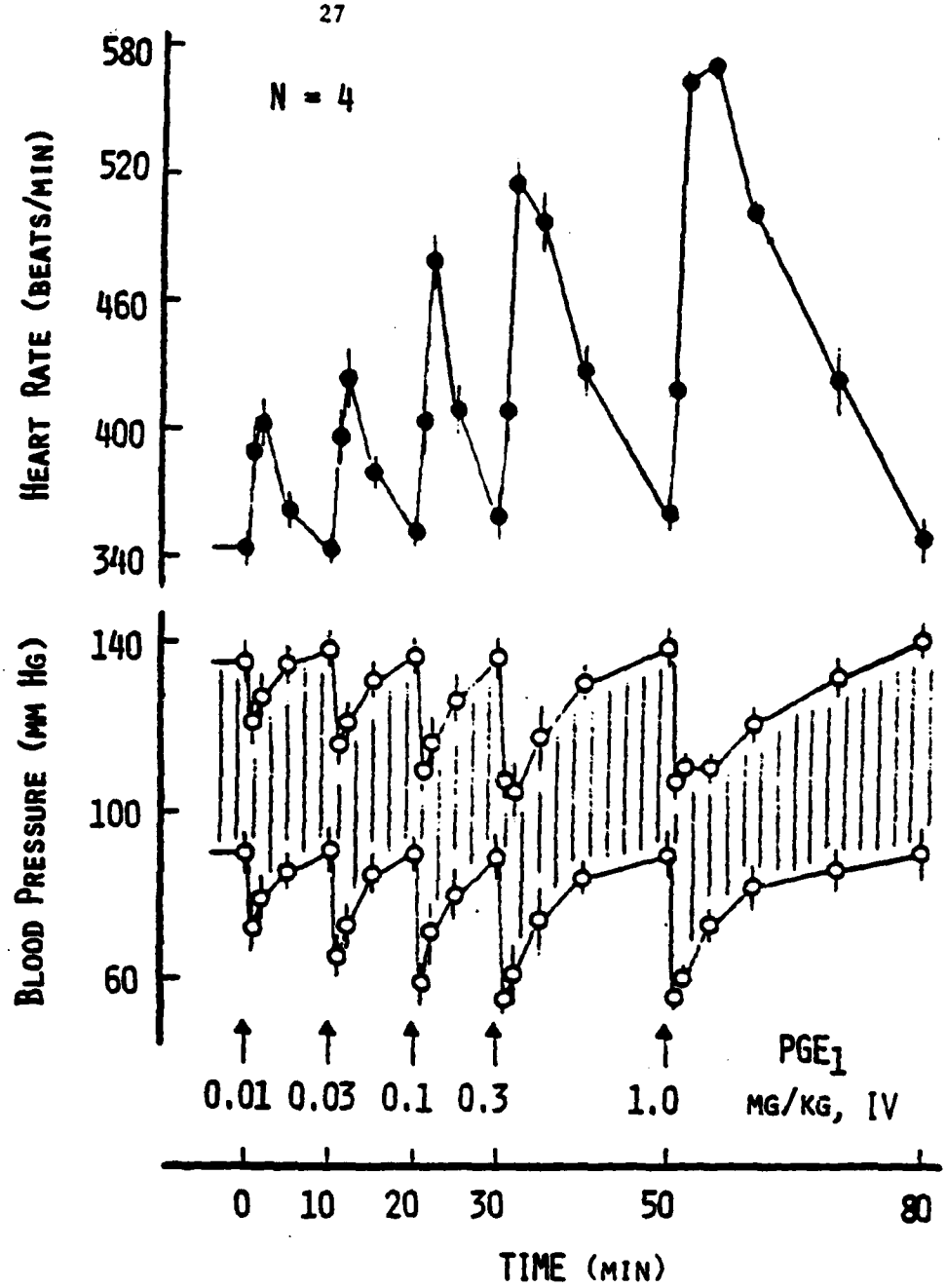


Fig. 14 Influence of PGE_1 (0.01-1 mg/kg, IV) on heart rate and arterial blood pressure of conscious, unrestrained rats. Transient tachycardia and hypotension are prominent.

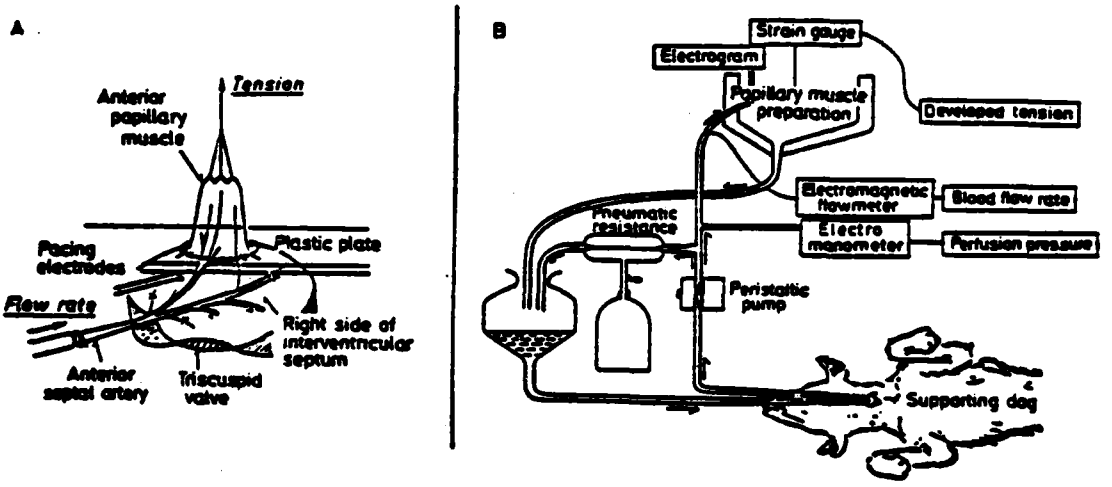


Fig. 15 Illustration of the papillary muscle preparation (A) and the cross-circulation system that supplies blood from a donor dog (B).

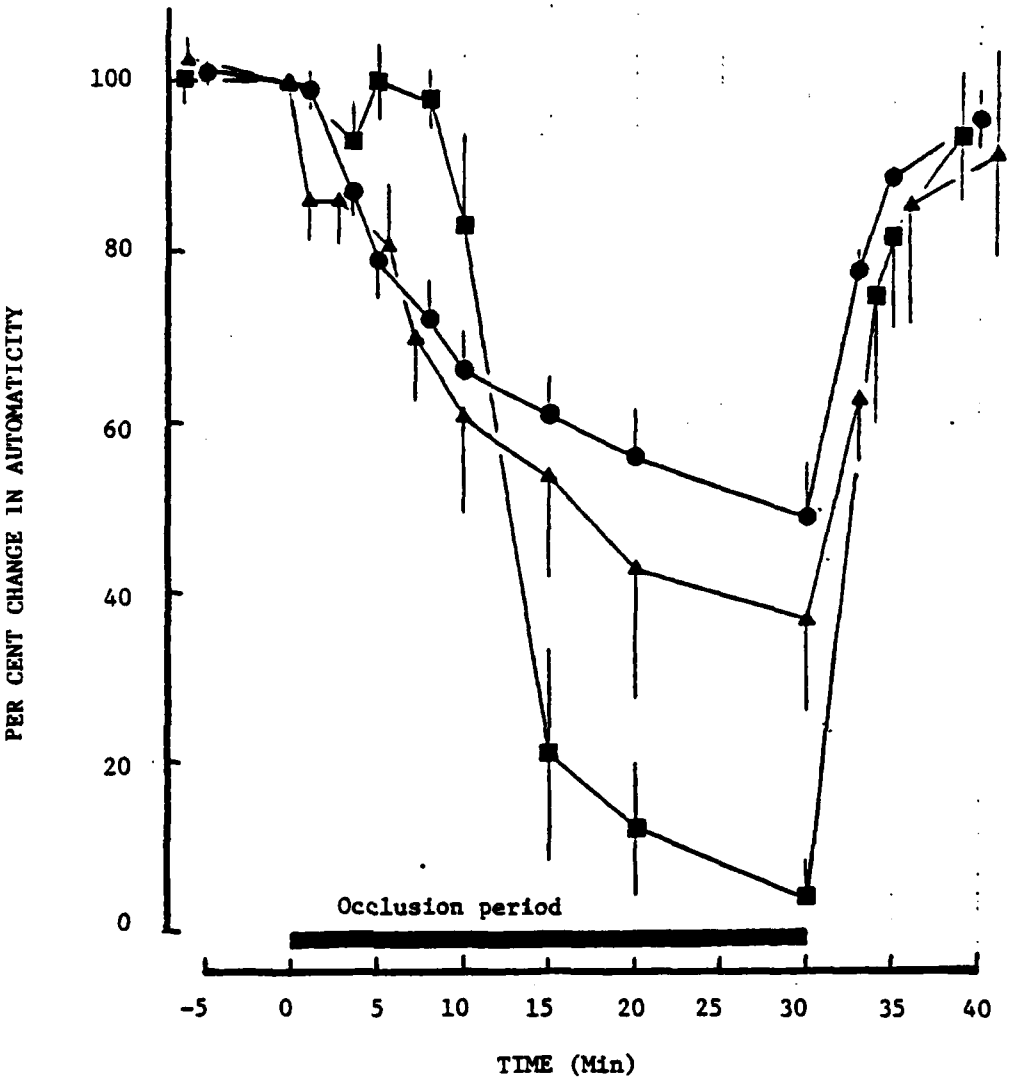


Fig. 16. Influence of 30 min arterial occlusion on the automaticity of the SA node (●), AV node (▲) and Purkinje fibers (■) of the canine heart, *in vitro*. Vertical bars represent SEM; N = 6.

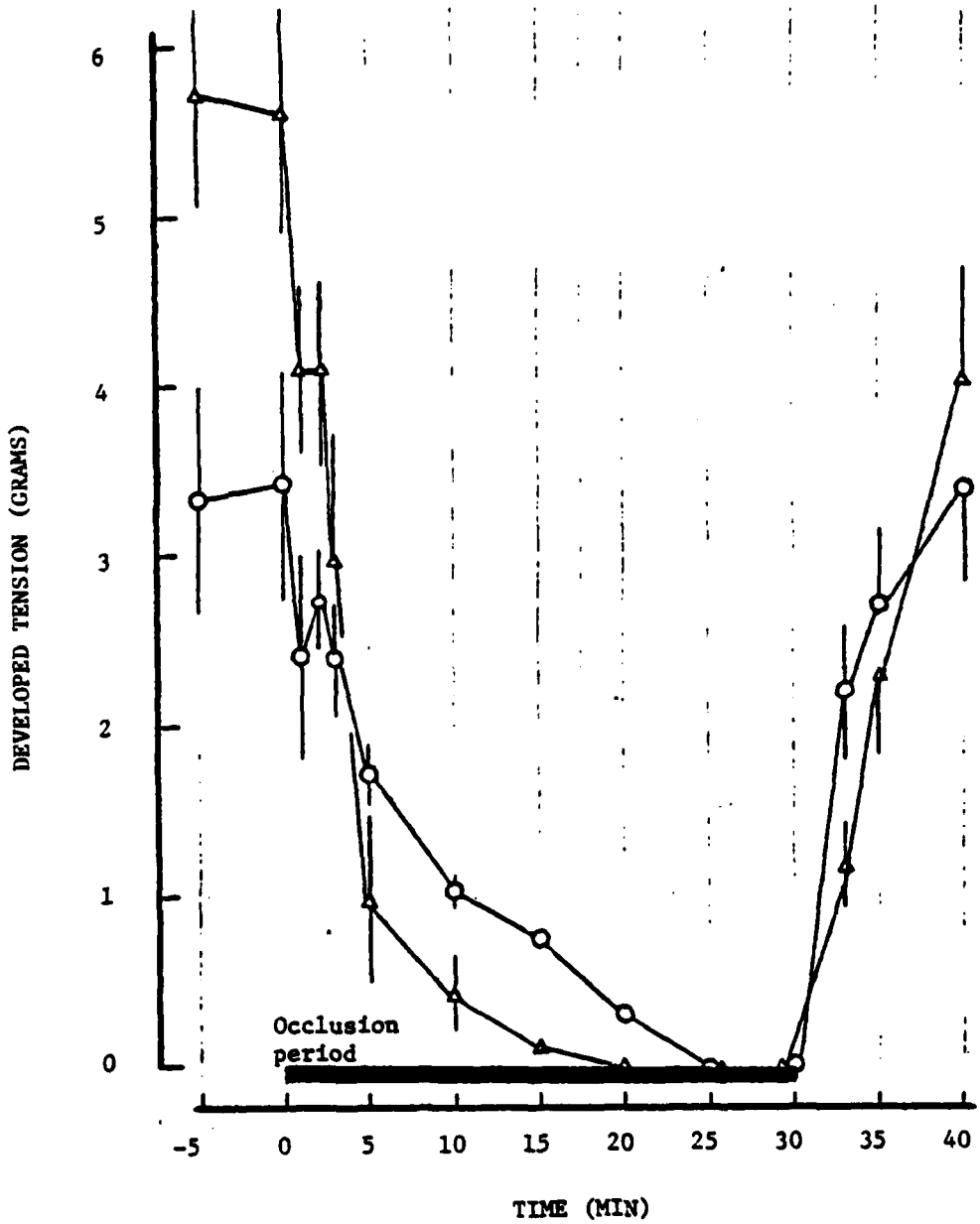


Fig. 17. Influence of 30 min arterial occlusion on the systolic tension developed in spontaneously beating (O) and electrically paced (Δ) papillary muscle. Vertical bars represent SEM; N = 6.

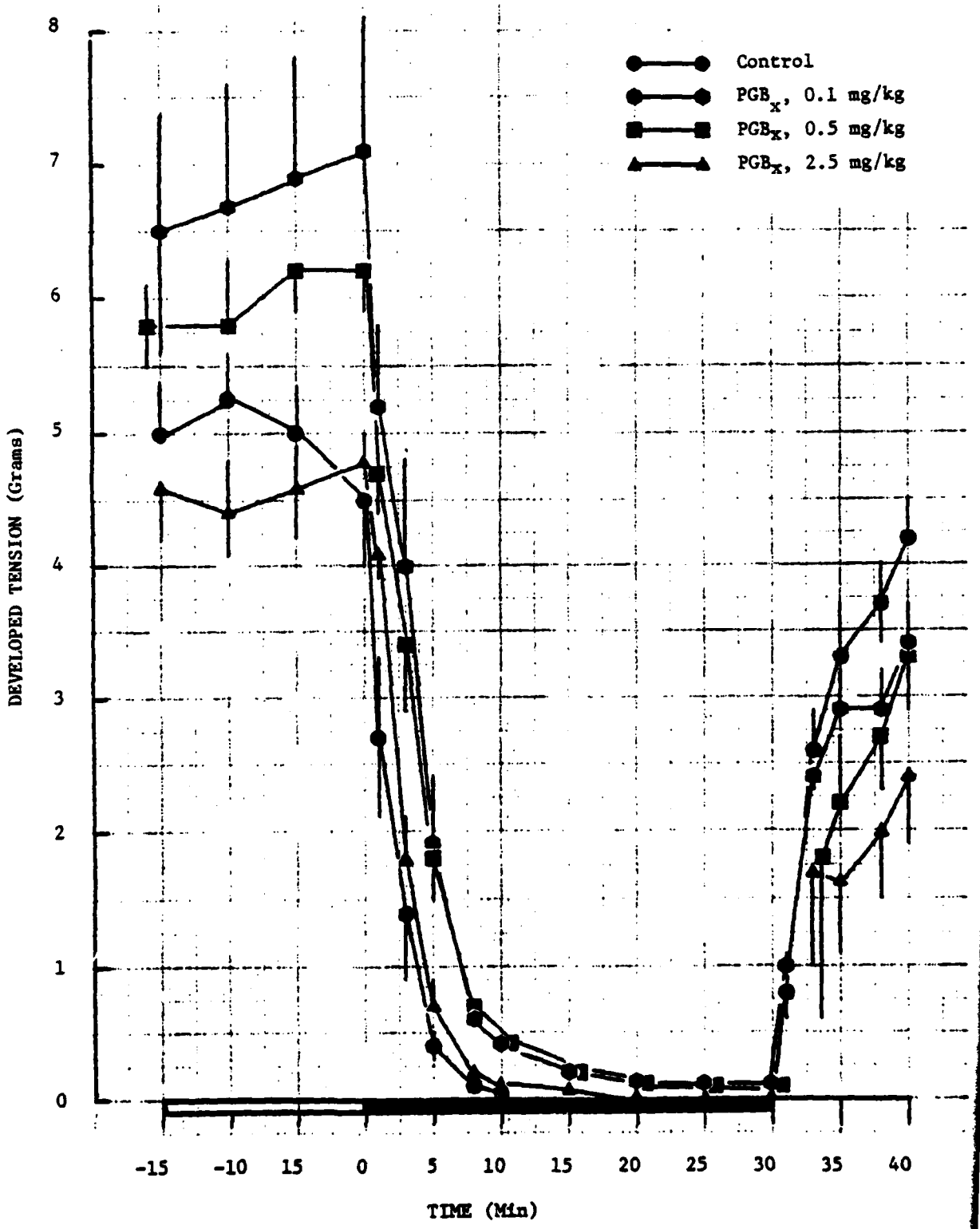


Fig. 18. Influence of PGB_x, administered intravenously into the support dog, on the systolic tension of oxygen deprived, electrically paced (2 Hz), papillary muscle. PGB_x was administered 15 min prior to arterial occlusion. N = 6.

APPENDIX A

Abstract of a paper submitted for presentation at the Eighth International Congress of Pharmacology, Tokyo, July 19, 1981 and for publication in the *Congress Proceedings*.

PROSTAGLANDIN B_X ENHANCES THE INOTROPIC EFFICACY (*Emax*) OF ISOPROTERENOL AND HISTAMINE ON ISOLATED GUINEA PIG RIGHT ATRIA. Allan M. Burkman and Srichan Phornchirasilp. Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, U.S.A.

Prostaglandin B_X (PGB_X), a mixture of oligomeric derivatives of 15-keto-PGB_1 methylester, exerts little or no influence on the inotropic or chronotropic behavior of spontaneously beating isolated right atria or electrically paced left atria. Pretreatment of right atria with PGB_X increases the maximum contractile force produced by both isoproterenol and histamine, an effect which is apparently not agonist specific. Although the variability in response among animals is large, this action of PGB_X has been adopted as the basis of an organ level bioassay used to evaluate PGB_X fractions, analogues and intermediates having variable degrees of biological activity. Components are examined for their ability to increase the positive inotropic effect of a fixed concentration of (-)-isoproterenol ($2x10^{-8}$M). The results are compared to a reference standard PGB_X preparation using a 4 point (2x2) parallel line design with 4 replications for each dose. The log dose response line of the reference standard has a dose-dependent range of 2-12 µg/ml bath, a slope of 38.53 percentage units/log dose unit, a correlation coefficient of 0.79 and an index of precision (lambda) of 0.258. The maximum increase of isoproterenol's effect produced by PGB_X is about 50% and using this value as the E_{max}, its ED50 was calculated to be 3.27 with 95% confidence limits of 2.70-4.00 µg/ml bath.

Supported by contract N00014-79-C-0122 from the Office of Naval Research.

APPENDIX B

Abstract of a paper presented at the Fall Meeting of the American Society of Pharmacology and Experimental Therapeutics, Rochester, Minn., Aug. 20, 1980, and published in The Pharmacologist, 22, 256 (1980).

Effects of PGB_x, PGB_1 and PGE_1 on the Pressor Responses to Electrical Stimulation of Sympathetic Outflow from the Spinal Cord of Rats. Norio Himori* and Allan M. Burkman, Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, OH 43210.

PGB_x, a synthetic polymeric derivative of PGB_1 was examined for its ability to alter vasomotor tone and heart rate in a) conscious unrestrained rats, b) adrenalectomized (ADX) pithed rats that had received exogenous norepinephrine, and c) ADX and pithed rats subjected to segmental cord stimulation. PGB_x (1-10 mg/kg, IV) had no significant effect on the B.P. or heart rate of conscious rats in contrast to PGE_1 (0.01-1 mg/kg, IV) which produced marked but transient hypotension and tachycardia. Bolus IV injections (10 mg/kg) and 7-day SC injections (2x1.2 and 2x6 mg/kg/day) of PGB_x significantly reduced the pressor response to electrical excitation of the cord in a time dependent manner. On the other hand, PGB_x inhibited norepinephrine-induced pressor effects to a much less dramatic degree. PGB_1 (10 mg/kg, IV) and PGE_1 (1 mg/kg, IV) produced short lived augmentation and inhibition, respectively, of the pressor response. Unlike PGB_1 and PGE_1, PGB_x ($1x10^{-5}$ to $3x10^{-4}$ g/ml bath) did not produce significant relaxation of guinea pig tracheal muscle. (Supported by ONR contract N00014-79-C-0122.)

APPENDIX C

Abstract of a paper to be presented at the Meeting of the Federation of American Societies for Experimental Biology, Atlanta, GA, April 12, 1980 and published in the Federation Proceedings.

PHARMACOLOGY

SUSCEPTIBILITY OF SPECIALIZED, CANINE CARDIAC TISSUE TO THE EFFECTS OF ISCHEMIA AS COMPARED TO CA^{++} BLOCKADE. Norio Himori*, Allen P. Walls* and Allan M. Burkman. Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, OH 43210.

We compared the effects of ischemia (30 min deprivation of blood supply) and Ca^{++} blockade on automaticity and contractility of isolated, canine sinoatrial node (SAN), atrioventricular node (AVN) and papillary muscle (PM) preparations cross circulated with blood from supporting dogs. Automaticity of SAN and AVN exhibited a high degree of resistance to ischemia, whereas automaticity and tension development of PM were more susceptible and were ultimately abolished. On the other hand, the Ca^{++} antagonist, SK&F 24260, exhibited little effect on PM automaticity but greatly depressed PM contractility and SAN automaticity leading to SAN arrest. Thus, it seems reasonable to suggest that acute deprivation of blood supply does not predominantly damage the functions of tissue in which the slow inward current is initiated by calcium ions. Furthermore, it was surprising that the total loss of contractility during ischemia was so readily reversible. Upon resumption of blood supply, the developed tension rapidly increased to the original pre-ischemic level. However, force-frequency analysis revealed that even after reperfusion the PM had lost the ability to respond normally to frequencies greater than 2 Hz. (Supported by ONR contract N00014-79-C-0122).

APPENDIX D

Abstract of a paper to be presented at the meeting of the Federation of American Societies for Experimental Biology, Atlanta, GA, April 12, 1980 and published in the Federation Proceedings.

PHARMACOLOGY

COMPARISON OF THE EFFECTS OF PROSTAGLANDIN B_X (PGB_X) AND VERAPAMIL ON CHANGES IN MYOCARDIAL FUNCTION THAT OCCUR DURING ISCHEMIA. Allen P. Walls*, Norio Himori*, and Allan M. Burkman. Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, OH 43210.

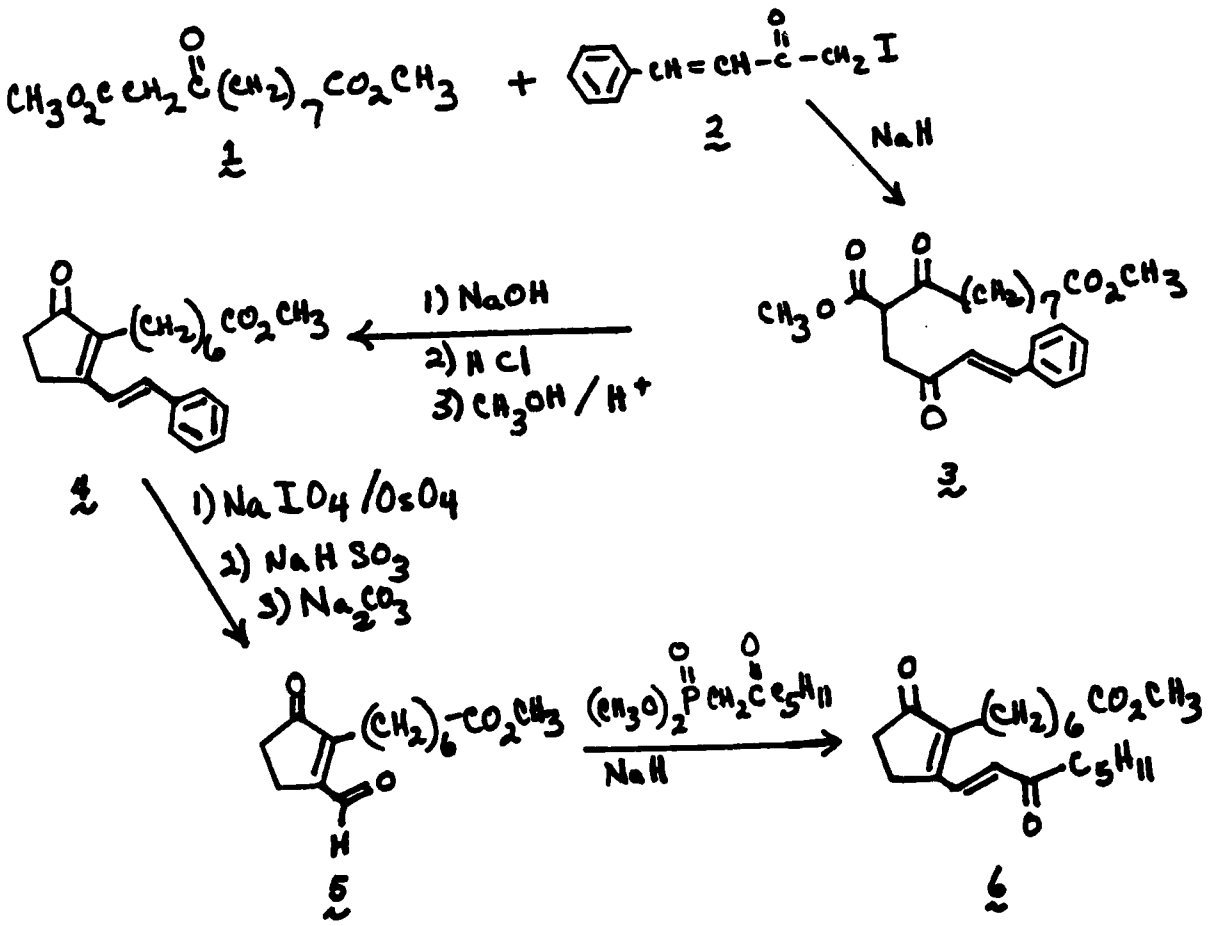
PGB_X, a synthetic oligomer of 15-keto-PGB_1, was examined for its ability to alter myocardial functions in both isolated canine sinoatrial node (SAN) and paced papillary muscle (2 Hz) cross circulated with blood from a supporting dog. Its actions, in the presence and absence of tissue ischemia, were compared with those of the calcium antagonist, verapamil. PGB_X (0.03-3 mg ia) slightly depressed SAN automaticity and papillary muscle contractility; 0.1-2.5 mg/kg injected iv into the supporting dog produced no significant effects on myocardial function but prevented papillary muscle standstill that usually resulted during a 30 minute occlusion of the perfusion artery; 0.1 mg/kg iv also prevented the ischemia-induced displacement of the force-frequency curve. In contrast, verapamil (0.4 mg/kg iv) exhibited negative chronotropic and inotropic actions and shortened the onset of ischemia-induced standstill. It also changed the force-frequency curve from one exhibiting positive force treppe with increasing stimulus frequency to one having negative characteristics. PGB_X, unlike verapamil, appears to directly protect the myocardium from some of the consequences of ischemia. (Supported by ONR contract N00014-79-C-0122).

Studies on the Synthesis of PGB_x

For the past year we have continued investigating new methods for the preparation of the methyl ester of 15-keto PGB_1. This substance is desired because we want to convert it to PGB_x so that composition and biological studies can be carried out on the resultant polymeric material. We are presently involved in finding an alternative shorter synthetic pathway which would allow for large scale production of the methyl ester of 15-keto PGB_1 and it is our objective to design a synthetic scheme that retains flexibility so that analogs of the methyl ester of 15-keto PGB_1 can be prepared. The synthetic scheme we have been working on for the past year is shown in Scheme 1. We are now capable of preparing the two starting materials dimethyl 3-oxoundecan-1, 11-dioate $\underset{\sim}{1}$ and 1-iodo-4-phenyl-3-buten-2-one $\underset{\sim}{2}$ in large quantities in routine fashion. The two known compounds $\underset{\sim}{1}$ and $\underset{\sim}{2}$ were combined to give the diester $\underset{\sim}{3}$ in good yield. Our main problem in the past and one that remains with us is the conversion of $\underset{\sim}{3}$ to $\underset{\sim}{4}$. In the past we have examined several pathways in an attempt to convert the diester $\underset{\sim}{3}$ to the cyclopentenone derivative $\underset{\sim}{4}$ (see the Technical Report No. 1 of April 30, 1980). We have recently found the shortest sequence for the cyclization and decarboxylation of $\underset{\sim}{3}$ to give $\underset{\sim}{4}$ involves treatment with dilute aqueous sodium hydroxide and work up followed by esterification with methanol. The sequence gave a mixture of products which are difficult to separate with preparative liquid chromatography using a silica gel column. We have had to carry out repeated chromatography steps in order to obtain pure $\underset{\sim}{4}$ in low yield. Once $\underset{\sim}{4}$ is isolated we convert it to aldelyde $\underset{\sim}{5}$ via the osmium tetroxide catalyzed periodate oxidation shown in Scheme 1. The aldelyde is then purified by sodium bisulfite and the adduct is decomposed to give $\underset{\sim}{5}$.

Compound 5 can be readily converted to 15-keto-PGB_1. We have found that compound 3 can be formed in large quantities but that the conversion of 3 to 5 does not go in an overall good yield and we have been attempting to improve this part of the sequence. Our latest attempt has been to add phenyl selenide to the α, β-unsaturated carbonyl system of 3 and then attempt cyclization to a five membered ring and then remove the phenyl

SCHEME 1



selenide group via oxidation to give 4. The initial reaction of phenyl selenide works well but the cyclization reaction has yet to provide the desired product. We are presently working with this sequence to attempt an improvement in the overall yield of 5. We are also looking presently at alternative routes to improve the yield of 5 so that we can prepare this material in large gram quantities.

We have examined the question of whether different types of bases used in the polymerization of the methyl ester of 15-Keto PGB_1 to PGB_x affect the biological activity of the final product. We used the standard reaction except we replaced the base normally used, potassium hydroxide, with an organic hydroxide (Triton B, N-benzyltrimethyl ammonium hydroxide) and with an amine (Dabco, 14-diazabicyclo [2.2.2] octane). The results in Table 1 indicate that the type of base used can affect the formation of active PGB_x.

TABLE 1[a]

Bioassay of the various bases used in the polymerization of the methyl ester of 15-Keto PGB_1

Base	µg/2.8ml	% Activity[b] 20	40	Solution	Comments
KOH		110	110	Clear	Same activity as standard
Triton B		90	110	Clear	Same activity as standard
Dabco		70	30	Hazy	Significantly lower activity than standard

a These results were obtained from Dr. T. Devlin, Department of Biological Chemistry, Hahnemann Medical College and Hospital.

b Activity as percent of the effect of an equal quantity of PGB_x (lot #27) to stimulate phosphorylation of rat liver mitochondria.

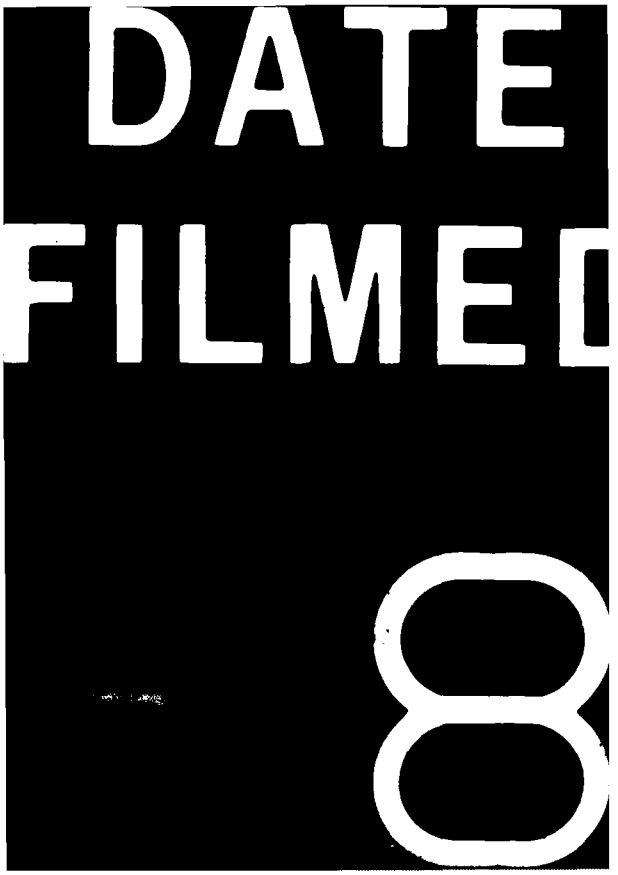
Studies on the Separation of PGB_x

In our Technical Report #1 which was up to April 10, 1980, the methods that were tried are given in detail. Only several other techniques could be applied since Dr. Sha'aban F. El-Naggar left the project April 30, 1980 for an appointment at the National Cancer Institute, Bethesda, Maryland. This was a serious blow to the project because he was a very gifted isolation chemist. His extensive experience in fractionation of complex natural products, gained during his dissertation studies, would in my judgement have yielded concrete results in the PGB_x investigation. His abrupt leaving was caused by pressure to be at NIH May 1, 1980, as the position could not be left vacant beyond that point. Although it was very unfortunate for the PGB_x study, the opportunity to extend his professional training was clearly the overriding factor. Since he was my graduate student and had already spent five years in my laboratory, I could not in good conscience prevent his departure. The vacant position was advertised on campus since nobody in the department was available. The announcement did not yield any applicants, undoubtedly because of the time of year. (Academic appointments generally end in June or later.) To add to the problem, we received a memo from Dr. R. Jennings which stated that his recommendation for the change in the direction of the PGB_x study would result in our project ending after this current year. It was only after assurances made to us at the Philadelphia meeting in July that this was not the case, that a vigorous effort was made to seek out a suitable research associate. It would be impossible to get someone for less than a year. This was done and a first-rate researcher, Dr. G. P. Dhareshwar from Bombay, India arrived and began his efforts on October 1, 1980. At the moment he is becoming familiarized with the techniques utilized by Dr. El-Naggar and shortly will be able to carry the study in new

directions.

The last few experiments performed by Dr. El-Naggar and finished by Dr. R. W. Doskotch involved partition column chromatography. This was a logical choice since paper chromatography appeared to yield some clues that partition chromatography may be of value. Separations were not spectacular but the conditions were not yet optimized. We plan to optimize them in the next year. Also, a droplet countercurrent chromatograph was purchased from Japan which will be utilized. Since it is basically a type of solvent-solvent partition system, we expect it to be an effective tool. Apparently some success with this equipment has been made at Columbia University. When the apparatus (ordered April 28, 1980) was received (June 18, 1980), there were a number of problems. First, the unit was for the European market (220 volts) while we required the 110 volt model. The company did not wish to replace it but instead sent a small transformer which when adapted caused fuses to blow. Eventually we got them to send us the 110 volt unit (received September 12, 1980) which had a defective pump and a long list of other shortcomings. However, in time the unit was made operational and has been, as of this date, checked through with a standard sample.

From the experience that Dr. El-Naggar had in which small samples of PGB_x mixtures were studied, we concluded the following: One, the PGB_x activity is most certainly not restricted to a single chemical entity. This is based on the fact that regardless of the type of separations we tried, the fractions all showed some activity (restoration of oxidative phosphorylation in aged mitochondria). Two, the mixture is highly complex, more so than any natural products mixture that we had ever encountered. This is understandable if you examine the precursor and type of reaction that is used to generate PGB_x. Clearly geometric, position and stereochemical isomers would be present. (Nature at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